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Anti-inflammatory effect of a long acting beta2-agonist (Salmeterol) and a corticosteroid (Fluticasone Propionate) on human airway epithelial cells following *Staphylococcus aureus* infection

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Staphylococcus aureus (*S. aureus*) is a major cause of pulmonary infection, frequently involved in early airway infection in CF patients. We investigated the effect of *S. aureus* soluble virulence factors (VF) on inflammation and junctional properties of human airway epithelial cells, and further analyzed the anti-inflammatory effect of Salmeterol (Sal) and Fluticasone Propionate (FP).
We first incubated the human tracheal glandular cell line (MM39) with Sal (2.10^{-7}M), FP (1.10^{-8}M) or their combination for 16 hours, then added a 20% supernatant culture of *S. aureus* strain 8325-4 for 0.5, 1, 2, 3 and 4h. IL-8, IL-6, TNFα and PGE2 release was measured by ELISA test on cell culture supernatants, IL-8 and TNFα mRNA expression by RT-PCR, NF-kB activity by EMSA, occludin and *Zonula occludens*-1 (ZO-1) protein expression by Western Blot and immuno-fluorescence.
From 0.5h up to 4h of interaction between *S. aureus* VF and MM39 cells, we observed a progressive degradation of occludin and ZO-1, associated with an increase ($P<0.001$) of IL-8 and TNFα mRNA expression, NF-kB activity ($P<0.05$) and IL-8, IL-6, TNFα and PGE2 release ($P<0.05$). In contrast, in MM39 cells pre-incubated with Sal and FP, we observed a decrease of IL-8 ($P<0.001$) and TNFα ($P<0.05$) mRNA expression after 1h of S. *aureus* VF incubation, associated after 3 h with a decrease ($P<0.05$) of IL-8, IL-6 and TNFα release.
These results emphasize the deleterious effect of *S. aureus* soluble virulence factors and demonstrate that Sal and FP attenuate the inflammatory response in airway epithelial cells.

Supported by INSERM, GlaxoSmithKline and Association Vaincre la Mucoviscidose.

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Expression of inducible nitric oxide synthase gene in airway epithelial cells in young children with CF

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Aims: This is the first study to measure inducible nitric oxide synthase expression quantitatively in primary epithelial cells from very young children with cystic fibrosis (CF).
Methods: Cells were obtained by tracheo-bronchial brushing in 40 children with cystic fibrosis (mean age ± SD, 2.1 ± 1.5 years) and 13 healthy, non-atopic controls (3.4 ± 1.2 years) and expression of iNOS was measured using quantitative PCR (TaqMan®) relative to the expression of β-actin.
Results: No difference in expression of iNOS was found in CF patients with and without bacterial colonization in broncho-alveolar lavage (BAL) (0.22 vs 0.23) however, iNOS expression was significantly lower in CF patients than in healthy children (0.75; $P=0.003$ for colonized and $P=0.009$ for non-colonized CF-patients). There was a borderline significant increase of iNOS expression with age in the CF patients, whereas gender, weight and genotype did not influence the expression of NOS. NOS expression was independent of the cellular components of BAL in the children with CF.
Conclusions: These results support the findings of previous studies in adult patients with advanced disease, cell lines and animal models. Our findings reflect the situation in young children without advanced disease. They indicate that low iNOS expression may be an innate defect with potentially important consequences for the local anti-microbial defense and suggest implications for a new therapeutic approach.

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Human airway trypsin-like protease modulates the urokinase receptor structure and functions

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Aims: The human airway trypsin-like protease (HAT) is a recently identified, type II transmembrane serine-protease endowed with a trypsin-like enzymatic activity. HAT which is synthetized by ciliated bronchial epithelial cells, can be detected as a major soluble active enzyme in the sputum of patients with chronic airway inflammatory disorders and might thus be involved in (patho)physiological mechanisms through the modulation of cellular and/or extracellular proteins.
Our project was to evaluate the capacity of HAT to alter the structure and functions of the 3-domains urokinase receptor (CD87/uPAR), which participates to innate immunity and inflammation by supporting cell migration and matrix degradation, and which can be regulated through endoproteolysis.
Methods and results: Through immunoblotting and ELISA analysis applied to a recombinant uPAR protein and to human bronchial epithelial cells as well as monocytes, we demonstrate that exposure to HAT results in the proteolytic processing of the intact (D1D2D3) uPAR into a truncated (D2D3) fragment. This processing modifies the receptor functions since removal of domain D1 makes the receptor unable to bind anymore two of its major ligands, the adhesive protein vitronectin and the serine-protease urokinase.
Conclusion: Such a proteolytic modulation of uPAR is likely to alter the motility of cells and the remodeling of tissues, and could thus participate to the regulation of the inflammatory response.

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Elastase-related proteases in cystic fibrosis sputum

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Unopposed proteolytic activity in sputum or BAL fluid is a characteristic feature of CF. Protease targeting by inhibitors appears therefore as an attracting strategy to treat inflammation and possibly bacterial proliferation in CF. Results however remained limited until now. One reason could be that efforts mainly focused on neutrophil elastase (HNE) and not on related proteases such as protease 3 (Pr3) and cathepsin G (Cat G) though these proteases are stored in similar amounts in neutrophil primary granules and are secreted at the same time by activated cells. The contribution of individual proteases to the pathogenicity in CF has not been evaluated because of the lack until recently of highly specific and sensitive substrates that allow to measure individual proteases activities in sputum or in BAL fluid of CF patients.
We raised a series of specific and sensitive substrates based on the S' specificity of these proteases (i.e. proteases subsites that accommodate substrate residues downstream the cleavage site). Those allow to discriminate between the activities of all three proteases, especially those of HNE and Pr3 that share very similar specificities. Proteases activities in the soluble phase and at the surface of activated cells in sputum were measured and the partition of each protease between the soluble and the solid phase was determined.
Since protease activities in the soluble fraction and in the solid phase could be differently regulated, we also measured the rates of inhibition of soluble and membrane-bound proteases by natural and recombinant inhibitors.
Discriminating between protease activities in CF sputum will be helpful to develop new inhibitors that appropriately control those of proteolytic activities that initiate, promote and make chronic lung inflammation and infection during CF.